



Confidential

Review of USB FFPW PhD Thesis

First name(s), surname, titles of the PhD student: Filip Ložek, Ing.	First name(s), surname, titles of supervisor: Prof. Ing. Pavel Kozák, Ph.D.,
Title of PhD thesis: Crayfish cardiac and locomotor activity as a tool for study of pharmaceutically active compounds effect	

REVIEWER:

Surname: Klobučar	Institution: Division of Zoology Department of Biology Faculty of Science University of Zagreb Croatia
Name: Goran	E-mail: goran.klobucar@biol.pmf.hr
Titles: Full professor	
Please describe your professional relationship to the PhD student: Senior colleague.	Please describe your field of expertise: Zoologist with keen interest in monitoring biological effects of pollution as well as in biology and phylogeography of freshwater crayfish.

QUESTIONNAIRE

Originality, scientific importance, perspectives and impacts of results presented in the PhD thesis for basic and/or applied research

Evaluate competitiveness of the PhD thesis in the international context and compare its level with the current state of the art in the field (**extent ¼ – ½ page**):

In the last decade or so there has been a renaissance in physiological/behavioural studies thanks to the advance in electronic and computer science. Research presented in this PhD thesis is part of this wave and, most importantly, has focused on crustaceans which have huge ecological importance as they represent a significant portion of the benthic biomass in freshwaters. The work presented here is both innovative and original.

Physiological/behavioural changes caused by pollutants, and especially pharmaceutically active compounds (PhACs) which represent a significant part of the emerging pollutants group, is highly important as pharmaceuticals and illicit drugs have become major part of organic pollution load in the freshwater environment.

Having in mind that this field of ecotoxicological research is currently experiencing expansion it is almost certain that such studies will be important in the future. The impact of the results presented in this PhD thesis is significant for both basic and applied research. Heart rate measurement in crayfish will help in better understanding of physiology and behaviour of crayfish as well as their responses to exposure to PhACs and illicit drugs that have become important pollutants in the



freshwater ecosystems.

Elaboration of the PhD thesis, objectives of the work and deliverables

Evaluate the overall level of elaboration of the PhD thesis (structuring of the main text, comprehensibility, logicity of the chapters and their ordering) and the originality of the selected approaches to solve the objectives; evaluate publications and whether the results described correspond to objectives of the PhD thesis (**extent ¼ – ½ page**):

This PhD thesis has been structured around four published papers dealing with measurement of the heart rate in freshwater crayfish (*Pacifastacus leniusculus*) in response to stress. After the general introduction to the problem of PhACs and especially psychoactive compounds in the freshwater environment and their impact on physiology/behaviour of invertebrates and the introduction of possible tools for studying such changes, four papers are presented where a logical structure/continuation is apparent. First paper describes the novel method of noninvasive measuring of crayfish cardiac activity along with the behavioural changes followed by the paper where the natural and chemical (disinfectant chloramine-T) stimuli are distinguished by crayfish in order to be able to study changes in responses to stress when exposed to environmentally realistic concentration of psychoactive compounds (Tramadol) what is covered in the third paper. Fourth and final paper (Chapter 5) describes the changes in the heart rate and behaviour (stress response) of crayfish as a result of exposure to 1 microgram per litre of illicit drug metamphetamine. The results of the presented papers do correspond to the objectives of the PhD thesis.

OVERALL COMMENTARY ON THE PhD THESIS

Please write comments in extent of 1-2 pages:

This PhD thesis covers a very exciting area of contemporary aquatic ecotoxicology that is important for environmental risk assessment: *in situ* measurement of physiological parameters (heart rate) and behaviour of freshwater crayfish that have a huge potential in future pollution biomonitoring. The idea of heart rate change as response to stress as a measurable end-point seems feasible. However, better quantification of a stress stimulus (scent of an injured conspecific) used for measuring response to a specific psychoactive compound in order to fully standardize the method, would be advisable.

Freshwater crayfish are usually absent from highly polluted aquatic ecosystems so this method is appropriate for better understanding of low or medium polluted freshwaters. This PhD emphasizes the importance of psychoactive compounds (mainly pharmaceuticals and illicit drugs but some pesticides have also neuroactive effects) for the behaviour of freshwater animals, specifically crustaceans. The most frequently measured and arguably the most important ecologically relevant individual-level adverse outcomes include survival, growth/development and reproduction. Other



types of individual-based effects that have high ecological relevance include behavioural changes, again because these often directly influence survival, growth and reproduction. And indeed, the PhD concludes that the crayfish organism with its biological functions is sensitive to detect selected psychoactive compounds in relatively low, environmentally realistic, concentrations. Furthermore, it also concludes that ethophysiological alterations highlight possible impacts of these compounds on the ecology of aquatic invertebrates. It is pity that additional research work (currently submitted) done by Filip and his colleagues was not included in this thesis as it covers the exposure of crayfish to a cocktail of antidepressants, anxiolytic, analgesic and illicit drugs. In rivers and lakes organisms are exposed to hundreds if not thousands of anthropogenic compounds (many of them PhACs and psychoactive). Even though these compounds typically occur at relatively low concentrations, their additive and synergistic contribution to toxicity, including the continuous chronic micro-exposure should be considered. Future research, based on the results of this PhD, will use wireless cardiac activity monitoring system what will enable study of the effects of selected PhACs on a wider range of behavioural patterns (also on juveniles!) within social or reproductive behaviour, in combination with cardiac activity. Hopefully long term studies will be also included as crayfish and other aquatic animals experience chronic microdosing with PhACs and illicit drugs and their metabolites, especially at their most sensitive stages (embryo and larvae), that might have negative effects on their development and behaviour lowering their reproduction success and survival.

Minor comments

In Table 2. species could be grouped by their taxonomical categories for each compound in order to be more clearly presented. At least some species could be placed together (f. ex. *G. pulex* and *P. virginalis*).

FINAL RECOMMENDATION

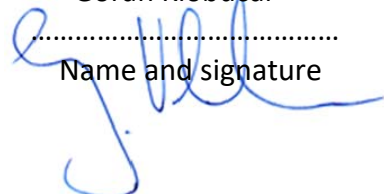
- PhD Thesis can be recommended for defence
 PhD Thesis can be recommended with reservations for defence
 PhD Thesis can not be recommended for defence

29-06-2020, Zagreb, Croatia

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Date and place

Goran Klobučar

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Name and signature